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Nitrogen Fixation in Arid Western Soils

Robert Charles Rychert
Utah State University

NITROGEN FIXATION IN ARID WESTERN SOILS

by

Robert Charles Rychert

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Approved:

Major Professor	Committee Member
Committee Member	Committee Member
Committee Member	Dean of Graduate Studies

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

ABSTRACT

Nitrogen Fixation in Arid Western Soils

by

Robert C. Rychert, Doctor of Philosophy

Utah State University, 1975

Major Professor: Dr. John Skujins
Department: Biology-Ecology

Nitrogen fixation by blue-green algae-lichen crusts from South Curlew Valley, Utah, in the Great Basin Desert, was studied using the acetylene reduction technique. A molar ratio of 3 moles C_2H_4 produced/mole of N_2 fixed was used to estimate nitrogen (N_2) fixation by acetylene reduction. Nostoc was found to be present in many of the lichen thalli examined microscopically. Crust nitrogen fixation decreased rapidly below -1/3 bar pressure (water potential) which indicated that nitrogen fixation occurs only when the crust is wet. This would suggest that most of the crust nitrogen fixation in the Great Basin Desert occurs during the fall and spring rainy seasons. Nitrogen fixation reached a maximum at 200 microeinsteins m^{-2} sec^{-1} of incandescent light intensity, somewhat comparable to natural light with a heavy grey cloud cover. In the winter months temperature would limit nitrogen fixation, and moisture would limit nitrogen fixation during the hot, dry summer months.

Ninety grams of nitrogen (N) per hectare was found to have been fixed in situ on a typical fall day, with peak in situ rates

of about 14 g N fixed ha^{-1} $hour^{-1}$. When assayed in the laboratory under optimal conditions (nitrogen fixation potential), crusts could fix nitrogen at rates as high as 84 g N fixed ha^{-1} $hour^{-1}$. Since moisture and light were not limiting in in situ assays, in situ nitrogen fixation correlated with predictions made from the laboratory determined temperature curve. There was some dark nitrogen fixation, with values varying from 5 to 30 percent of nitrogen fixation in the light. An estimate of 10 to 100 Kg N fixed ha^{-1} $year^{-1}$ was made, based on 120 days of fixation. The absolute value would vary from area to area depending upon the extent of blue-green algae-lichen crust cover, and with temperature, moisture availability, and duration of the rainy periods.

Glucose was found to potentiate crust heterotrophic nitrogen fixation with an optimum at 30 C. Soils beneath the crust exhibited no acetylene reduction, unless amended with glucose, and soils at the 40 to 50 cm depth showed no ability to reduce acetylene even when amended with glucose. Apparently the supply of available organic carbon in the soils is insufficient for significant heterotrophic nitrogen fixation to occur.

Ammonium ion was found to inhibit crust nitrogen fixation, pointing toward ammonium ion regulation of nitrogen fixation.

May versus September, 1973 crust transect samples from sites dominated by Atriplex confertifolia, Ceratoides lanata, and Artemisia tridentata were not significantly different. However, the Ceratoides lanata values were significantly lower and reflected the scant lichen cover in that desert shrub community. Nitrogen fixation was found to be greatly reduced under the canopies of the three desert

shrubs mentioned previously, with an understory dominance by bryophytes. Aqueous leaf extracts and leaf volatile products inhibited crust nitrogen fixation, with the inhibition being particularly pronounced with Artemisia tridentata leaf extracts. Glucose was used to potentiate heterotrophic nitrogen fixation. Dried plant leaves, when added to soils, and the mixed soil and leaves moistened with glucose solutions, inhibited heterotrophic nitrogen fixation. The heterotrophic nitrogen fixation potential (glucose amended soils) was greatly reduced in soils from beneath the desert shrub canopies. Thus, shrub inhibitors may play a role in nitrogen input and blue-green algae-lichen crust distribution in desert shrub communities.

(78 pages)

INTRODUCTION

Nitrogen fixation by desert algal crusts probably constitutes a major input of nitrogen into desert ecosystems (Fuller, Cameron, and Raica, 1960; MacGregor and Johnson, 1971; Mayland, McIntosh, and Fuller, 1966). The nitrogen fixed by the algal crust is available to desert plants (Mayland and McIntosh, 1966). However, there have been no studies of nitrogen fixation in arid soils that assessed the environmental and ecological parameters affecting biological nitrogen fixation. Most of the studies of biological nitrogen fixation have been with more temperate ecosystems (Hardy, Burns, and Holsten, 1973; Hardy et al., 1968).

This study describes the moisture, temperature, and light intensity parameters that govern blue-green algal-lichen crust nitrogen fixation in South Curlew Valley, Utah, in the Great Basin Desert. Since its discovery by Dilworth (1966) and Schollhorn and Burris (1966), the acetylene reduction technique has proved to be a useful and valid method for estimating biological nitrogen fixation (Hardy et al., 1968; Stewart, Fitzgerald, and Burris, 1967). The acetylene reduction technique is used here to estimate nitrogen fixation potentials in the laboratory which are compared with nitrogen fixation under field conditions. Soil heterotrophic nitrogen fixation and the potentiation of heterotrophic nitrogen fixation are also examined.

Nitrogen fixation is also examined in relation to three desert shrub communities: a sagebrush (Artemisia tridentata) dominated

area; a winterfat (ceratoides lanata) dominated area; and a shadscale (Atriplex confertifolia) dominated area.

Some chemicals released by plants may be inhibitory or toxic to soil microorganisms and may exert allelopathic effects in this fashion (Whittaker, 1970), and allelopathic effects have been suggested to be more common in arid regions (Went, 1970). Thus, the relationship of blue-green algal-lichen crust nitrogen fixation to the individual desert shrubs is examined since plant inhibitory phenomena could limit nitrogen input. However, autoallelopathy, allelopathy, and related inhibitory effects might provide a selective advantage to plants in an arid ecosystem where nutrient supply to the standing biomass is already limited.

REVIEW OF THE LITERATURE

Biochemistry and determination of nitrogen fixation

The biochemistry of nitrogen fixation has been well reviewed by Bergersen (1971), Dalton and Mortenson (1972), and Hardy, Burns, and Holsten (1973). Nitrogen (N_2) is reduced to ammonia by the nitrogen-fixing enzyme complex (nitrogenase). The nitrogenase catalyzes ATP-dependent reductions of H^+ and six classes of molecules containing triple-bonded carbon and/or nitrogen atoms. Both Mo and Fe are present in nitrogenases with the Fe being involved in the binding of N_2, and Mo being involved in reducing the strength of the nitrogen bonds so that reduction can be facilitated. However, nitrogen fixation cannot be considered an isolated metabolic event since it is integrated with energy-yielding metabolism, electron transport, ammonium assimilation, and cell growth.

Nitrogen fixation has been assayed using Kjeldahl nitrogen determinations, fixation of $^{15}N_2$, and by the acetylene reduction technique. The ^{15}N method is 10^3 times as sensitive as Kjeldahl methods, and the acetylene reduction technique is 10^3 to 10^4 times as sensitive as ^{15}N methods (Hardy, Burns, and Holsten, 1973).

The acetylene reduction assay is particularly valuable for *in situ* assays (Stewart, Fitzgerald, and Burris, 1967), and has been applied to both laboratory and *in situ* studies with a diversity of biological nitrogen-fixing systems (Hardy et al., 1968). There have been a number of variations for field studies using serum

bottles, mason jars, and Pankhurst tubes (Campbell and Evans, 1969; Stutz and Bliss, 1973; Waughman, 1971).

Acetylene serves as a useful internal standard in both laboratory and in situ assays since, with most assays, only a small fraction of acetylene is reduced to ethylene. It may be important however, to verify the values for nitrogen fixation obtained by acetylene reduction with $^{15}N_2$ fixation methods, since variability may exist in field versus laboratory conditions (Bergersen, 1970). For most studies using the acetylene reduction technique, a theoretical ratio of 3 moles of C_2H_2 reduced per mole of N_2 fixed has been assumed. Average ratios of 3.6, 4.3, and 3.2, have been obtained for bacterial enzyme (nitrogenase) preparations, bacteria, and blue-green algae, respectively (Hardy, Burns, and Holsten, 1973).

The kinetics of acetylene reduction are such that the Km for C_2H_2(average for all biological systems was 0.006) is much greater than that for N_2. Thus, a concentration of C_2H_2 of 0.1 atmospheres in assays eliminates any N_2 fixation (Brouzes and Knowles, 1973; Hardy, Burns, and Holsten, 1973; Stewart et al., 1971).

The acetylene reduction assay can also be used to assess phosphorus availability in lakes (Stewart, Fitzgerald, and Burris, 1970).

Microorganisms and nitrogen fixation

Nitrogen fixation by Rhizobium in association with legume root nodules is an important source of nitrogen (Dalton and Mortenson, 1972). However, root-nodule formation and nitrogen fixation does occur in many non-leguminous plants (Bond, 1971). Nitrogen fixation

by free-living bacteria like Azotobacter and Clostridium is limited by the amount of available organic carbon. Of the free-living forms the blue-green algae are probably the most important contributors of fixed nitrogen (Dalton and Mortenson, 1972; Stewart, 1971).

Heterocystous, filamentous blue-green algae possess a nitrogenase and can fix N_2 under aerobic and micro-aerophilic conditions. Most studies have involved Anabaena and Nostoc species. Non-heterocystous, filamentous blue-green algae (e.g., Oscillatoria and Plectonema), may fix nitrogen micro-aerophilically; whereas unicellular, non-heterocystous Gloecapsa may fix nitrogen aerobially and micro-aerophilically (Fogg et al., 1973).

Blue-green algae generally grow well under reducing conditions as in water-logged soils, and cell free extracts of the nitrogenase of blue-green algae are very sensitive to oxygen inhibition. However, intact cell nitrogen fixation is inhibited at oxygen concentrations above 20 percent (Stewart, 1971). DCMU (dichlorophenyl dimethyl urea) does not inhibit nitrogen fixation completely in blue-green algae, indicating that photosystem II, the oxygen evolving system is absent from heterocysts. Thus there would be little photorespiration in heterocysts and negligible loss of reductants. Cyclic photophosphorylation is assumed to supply the ATP needed for nitrogen fixation (Stewart, 1973). However, Benemann and Weare (1974) and Henriksson et al., (1972) have reported significant amounts of acetylene reduction by blue-green algae in the dark.

Wolk and Wojciuch (1971) have shown that heterocysts of aerobically grown Anabaena cylindrica have a capacity to reduce

nitrogen at a rate much in excess of the average per-cell rate of fixation by intact filaments, although only 30 percent of the nitrogenase activity was recovered when heterocyst suspensions were prepared from intact filaments. Heterocysts lost activity rapidly under aerobic conditions, implying that the structural relationship between vegatative cells and heterocysts is important in protecting the nitrogenase from oxygen inactivation.

TTC (triphenyl tetrazolium chloride) reduction may prove useful in assaying nitrogenase activity within heterocysts. TTC inhibited 75 to 78 percent of acetylene reduction with an Anabaena cylindrica culture (Stewart, Haystead, and Pearson, 1969). Heterocyst ^{15}N nitrogen was rapidly transferred to vegetative cells.

An inverse correlation has been found between the level of free ammonia in the intracellular pool of Anabaena cylindrica, and the level of acetylene reduction. Ammonium ion may then regulate the level of nitrogenase in vivo, but it appears that glutamine/glutamate synthetase pathways convert ammonia rapidly into organic compounds (Dharmawardene and Stewart, 1972).

Glucose can potentiate heterotrophic nitrogen fixation, however in situ, the supply of readily-assimilated organic carbon is presumably too low. Both glucose and mannitol could increase nitrogen fixation in sandy loam field soils. Higher anaerobic efficiencies were obtained, and it was suggested that under aerobic conditions, there was an intense competition for available carbohydrate (O'Toole and Knowles, 1973a, 1973b).

Addition of organic matter (as 5 percent ground leaves) or

2 percent glucose or sucrose, significantly increased acetylene reduction of soils from Western Nigeria, with anaerobic soils exhibiting more acetylene reduction than aerobic soils. It was suggested that the low or negligible rate of acetylene reduction with unamended soils was due to a lack of decomposable (available) organic carbon (Spiff and Odu, 1972).

The nitrogen-fixing blue-green algae Nostic, Scytonema, Calothrix, Stigonema, and Dichithrix may occur in lichens. Photosynthetically fixed carbon is rapidly transferred to the fungus with mycobiont respiration possibly encouraging low pO_2 levels. Blue-green algae may also be associated with bryophytes, pteridophytes, gymnosperms, and angiosperms (Fogg et al., 1973).

Bond and Scott (1955) found that the lichens Collema granosum and Leptogum lichenoides both, had Nostic as a symbiont and could fix $^{15}N_2$. Nostoc associated with the liverwort Blasia pusilla could also fix $^{15}N_2$.

Henriksson et al., (1972) calculated that the blue-green algae in some Swedish soils could fix up to 330 kg N per hectare per 1000 hours. The range was 0.01 to 4.5 mg N / m^2 / hr. Nitrogen fixation occurred during the day and night and appeared independent of temperature. However, Balandreau, Millier, and Dommergues (1974) found diurnal fluctuations in nitrogenase activity in a rice field, grassland, and peanut field. These results suggest that environmental parameters do influence nitrogen-fixing systems.

Scott (1956) found that the lichen Peltigera praetextata (with a Nostoc symbiont) could fix $^{15}N_2$. Azotobacter did not contribute to

lichen $^{15}N_2$ fixation. Peltigera aphthosa could fix $^{15}N_2$, at 25 C, at a rate of 3.6 ug N / day / ug algal N (Milbank and Kershaw, 1969). They concluded that, in general, lichens that fix nitrogen appear to have Nostoc as the algal symbiont. Milbank (1972) found that the nitrogenase activity of the algal cells was sensitive to disturbance (cutting) of the thallus of Peltigera canina. Nostic cells separated by density gradient centrifugation showed no acetylene reduction. It was implied that the mycobiont reduces the light intensity and oxygen tension over the algae.

Microorganisms and nitrogen fixation
in arid ecosystems

While both blue-green and eucaryote algae are found in the surface crusts of deserts (Lynn and Cameron, 1971), it is some of the blue-green algal species that are responsible for biological nitrogen fixation. The great majority of blue-green algae that fix nitrogen are probably heterocystous, but non-heterocystous blue-green algae may fix nitrogen as well (Stewart, 1973). Plectonema borynum (non-heterocystous) was shown to fix nitrogen micro-aerophilically, but not in air (Stewart, 1971). Micro-environmental conditions where oxygen tension is reduced might enhance nitrogen fixation by both non-heterocystous and heterocystous blue-green algae. Desert lichen crusts might then provide a situation where mycobiont respiration could reduce oxygen tension so that nitrogen fixation might be enhanced.

While there has been a great deal of work involving nitrogen fixation by blue-green algae and lichens from temperate regions

(Henriksson, Enckell, and Henriksson, 1972; Henriksson and Simu, 1971) and with aquatic systems (Stewart, 1973), the significance of nitrogen fixation by blue-green algal and lichen crusts in desert ecosystems has received limited attention.

Desert algal crusts are found on neutral to alkaline soils. Such sonoran desert algal crusts were found to have a net N_2 fixation rate of 0.16 lb / acre-day under continuously wet conditions, and 0.10 lb / acre-day under cycling wet-dry conditions, using ^{15}N methodology (Mayland, McIntosh, and Fuller, 1966). N_2 fixation rates increased linearly for 520 days in laboratory studies. Growing algal crusts excreted 1 to 2 percent of the total crust N as extracellular NH_4^+ - N. Grass seedlings could take up some of the fixed $^{15}N_2$.

MacGregor and Johnson (1971) found that Sonoran desert algal crusts from southern Arizona could produce detectable ethylene from acetylene three hours after moistening. Premoistened algal crusts could produce 78 ± 5 nanomoles of ethylene cm^{-2} per hour based on the first hour of incubation. It was estimated that following a rainfall, 3 to 4 grams of N / ha-hr might be fixed. Approximately 4 percent of the surface of an area of desert grassland had crust formations. Most of the nitrogen fixation probably took place during the Sonoran desert's summer rains.

Mayland and McIntosh (1966) showed that algal crust-fixed $^{15}N_2$ was available to higher plants; 3.4 percent of the crust nitrogen was water soluble, and 1 percent of the crust nitrogen was NH_4^+ - N.

Fuller, Cameron, and Raica (1960) showed that blue-green algal and lichen crusts from the Sonoran desert could fix $^{15}N_2$, and that this N was subsequently available to plants. Sonoran desert algal crusts were higher in nitrogen and carbon than subsurface layers (Cameron and Fuller, 1960). Since Azotobacter were absent, nitrogen fixation by blue-green algae was assumed. Species of Nostoc, Scytonema, and Anabaena were shown to fix atmospheric nitrogen. There was great variation of algal development in laboratory cultures with varying environmental conditions, and thus it was difficult to identify any particular alga. Blue-green algae from the Negev Desert have been difficult to culture and identify (Friedman, Lipkin, and Ocamp-Paul, 1967).

Rogers, Lange, and Nicholas (1966) found that the lichen Collema coccophorus (with a Nostoc phycobiont), from the south-central arid zone in Australia, could incorporate significant amounts of $^{15}N_2$.

Skujins and West (1973) have shown that $^{15}N_2$ could be fixed, in situ, by blue-green algal-lichen crusts in the Great Basin Desert. Spring in situ assays showed that the highest period of N_2 fixation occurred during the morning hours. The highest in situ peak value was approximately 10 g ^{15}N fixed / ha / hr, and occurred between 8:00 and 10:00 a.m. It was suggested that morning dew condensation on the surface crust could provide sufficient moisture for crust nitrogen fixation.

Algal rain crusts in the Sonoran desert hold soil particles and reduce erosion. Soil fertility was increased. Soil structure

and water infiltration were also improved. Oscillatoria, Nostoc, Microcoleus, and Nodularia algal species were present in the surface crusts. No Azotobacter were found. The crust contained 400 percent more nitrogen than the surface below (Fletcher and Martin, 1948). The presence of cryptogamic crusts on the Colorado Plateau resulted in increased water infiltration rates (Loope and Gifford, 1972).

Microcoleus vaginatus, Schizothrix californica, and S. acutissima have been observed upon microscopic examination of Nevada desert crusts. Nostoc commune and Scytonema hofmanii were found to be associated with lichens (Shields and Drouet, 1962). The surface 0.5 inches had nitrogen levels twice as high as areas where there was no lichen crust. Mosses (Grimmia) grew on the upper part of the hummock beneath plant canopies, and lichens developed at the lower part where the water drains. It was suggested that mosses appear in the more xeric environment. While the blue-green algae are resistant to dessication, the Chlorophyceae are not.

Cameron (1962) found species of Nostoc which had a wide Sonoran desert distribution. N. muscorum along with Scytonema hofmanii were common constituents of lichen crusts. N. microscopicum and N. ellipsosporum were found associated with mosses. The lichens had a high resistance to dessication and moisture was retained for a greater period of time than in surrounding soil. Air-dried algal and lichen crusts were revived after four years of dessication. After one year dessication, there was no change in growth compared to original growth experiments. Apparently, survival of Nostoc

muscorum does not depend upon sporulation.

Cameron and Blank (1966) have found that desert algal-lichen soil crusts can survive, grow, and reproduce in cultures after subjection to extreme cold. This ability would adapt the soil algal microflora to harsh environmental conditions.

Lynn and Cameron (1971) obtained 4.0×10^4 nitrogen-fixing bacteria per gram of soil for Curlew Valley, the US-IBP Great Basin Desert site; whereas New Mexicl and Arizona desert soils had nitrogen-fixing bacteria roughly ten times higher than the Curlew Valley site (Lynn and Cameron, 1972). Percent algal cover in various Curlew Valley desert shrub communities varied from 41 to 82 percent depending upon the plant community (Lynn and Cameron, 1973). However, no data are yet available regarding extent of cover by nitrogen-fixing blue-green algae and lichens.

The free-living nitrogen-fixing bacteria, Azotobacter and Clostridium, are present in relatively high numbers in soils from the Near East except where the soil was bare or where salinity was high (Abd-El-Malek, 1971). With cropping or organic matter (maize stalks, wheat straw) addition, along with irrigation, significant increases were seen in the number of nitrogen fixers along with increases in soil nitrogen content. Less nitrogen was gained in calcareous soils than in clay soils. In the absence of organic carbon ammendments, heterotrophic nitrogen fixation was negligible. However, micro-environmental conditions, such as those occurring in the rhizosphere and within blue-green algal and lichen crusts, could provide situations where there is a transient supply of available

carbon. Azotobacter may be present and fix nitrogen in desert lichen crusts, but definitive evidence is lacking (Snyder and Wullstein, 1973a). Nostoc was associated with the lichens Dermatocarpon lachneum and Peltigera refescens, and Azotobacter nitrogen fixation was implicated with the moss Grimmia. Snyder and Wullstein (1973b) did find Azotobacter associated with Georgia granitic pioneer outcrop systems (lichens), and suggested that nitrogen fixation by Azotobacter may contribute to the nitrogen budget of that ecosystem.

While the primary input of nitrogen in desert ecosystems would appear to be nitrogen fixation by blue-green algae, there is evidence of root nodule formation and nitrogen fixation with Artemisia ludoviciana (Farnsworth and Hammond, 1968).

Nitrogen fixation and desert shrub communities

Many plants produce allelopathic chemicals, substances toxic to the germination and/or development of other plants, and often to members of the same species. Some chemicals released by plants are inhibitory or toxic to soil microorganisms and may exert their allelopathic effect in this fashion (Whittaker, 1970).

There are a number of means by which a plant can release a toxic chemical. Release may be from the leaf by dewfall and rain-wash (leachate), by litter fall, or by abiotic and biotic decomposition by-products of litter and/or leachate. There is also evidence of volatile inhibitors being released (Muller, Muller, and Haines, 1964). Inhibitory root exudates or root exudate microbial decomposition products would have to be primarily effective against microbial

plant pathogens (Cruickshank and Perrin, 1964), but underground root inhibitory effects are apparently responsible for "fairy rings" in Helianthus (Muller and Muller, 1956).

Chemical data would indicate that allelopathics are largely phenolic or terpenoid, with the phenolics assuming more importance in moist habitats (Whittaker, 1970). Muller (1965) has identified cineole and camphor as particularly potent allelopathic terpenes produced by Salvia leucophylla. Bacterial numbers were reduced in the soil beneath Salvia but were higher under adjacent grass communities. Muller and del Moral (1966) found that soil exposed to Salvia vapors retained its toxicity toward cucumber seedling germination for several months. More toxin was adsorbed to colloids in the "dry" state. On the other hand, the allelopathy of Encelia farinosa toward a number of plants was particularly apparent with coarse gravel soil, where adsorption and microbial activity would be low (Muller and Muller, 1956). Adsorption to clays can result in an increase or a decrease of microbial decomposition of an organic compound (McLaren and Skujins, 1968), with a subsequent effect upon the persistence of that compound.

Root exudate microbial inhibitors would have to be somewhat specific since, in virtually all groups of microorganisms examined, the absolute number of microorganisms of a group increases within the rhizosphere (Hattori, 1973).

Extracts from plants involved in Oklahoma old field succession inhibit nitrogen-fixing (Azotobacter) and nitrifying (Nitrosomonas, Nitrobacter) bacteria, and it was suggested that this inhibition

was responsible for the success of various stages (Rice, 1964). The inhibitors were phenols, polyphenols, and gallotannins, and could be adsorbed by kaolin (Rice, 1965). Bromus janicus and other pioneer stages demonstrated differential toxicity with respect to plant age (Rice, 1967). Rice and Pancholy (1973) have shown that tannins from climax species in Oklahoma old field succession could inhibit nitrifying bacteria. The concentration of tannins found in climax soils was several times that necessary to completely inhibit nitrification.

Allelopathic chemicals have been suggested to be more common in arid regions (Went, 1970). Since significant microbial decomposition activities is restricted to relatively short, wet periods, persistence of allelopathic chemicals might be expected. Auto-allelopathy is also particularly strong in plants from arid regions (Muller, Muller, and Haines, 1964; Whittaker, 1970).

The environment beneath desert shrub canopies receives plant litter and leachate from the plants. In the Great Basin desert, the microenvironment beneath the canopies of Artemisia tridentata, Ceratoides lanata, and Atriplex confertifolia is largely dominated by bryophytes (Skujins and West, 1973). In the interspace between the shrubs, a blue-green algal-lichen crust dominates with few or no bryophytes. Shrub litter and leachate may then play a role in nitrogen fixation and other nitrogen transformations, particularly in the canopy microenvironment.

MATERIALS AND METHODS

Sample collection

Soil cores, 3 cm in depth, with an intact blue-green algal-lichen crust, were collected in 13 x 60 mm glass tubes from sites dominated by sagebrush (Artemisia tridentate; site no. 5), winterfat (Ceratoides lanata; site no. 6), and shadscale (Atriplex confertifolia; site no. 7), in South Curlew Valley, Utah, in the Great Basin Desert. The cores were sealed at the bottom with a rubber stopper and at the top with an injectable serum bottle rubber stopper. Cores were stored at 3 C until use. Cores collected "dry" could be stored at room temperature (21 $\pm$ 2 C) until use, without loss of activity.

Intact soil crusts (0-3 cm soil depth) were collected from the three sites mentioned above, in sterile whirl-a-pak bags, and stored at 3 C until use. Soil samples were collected at 0-3 cm, 5-20 cm, 40-50 cm, and 70-8- cm depths from the three experimental sites. The samples were collected in sterile whirl-a-pak bags and stored at 3 C until use. Each sample was mixed well with a mortar and pestle prior to use.

Area description

The experimental area receives about 18 cm precipitation annually and the vegetation is representative of the valleys within the Great Basin region. The physical properties of the soils in relation to the prevailing desert shrub communities in experimental area are described in Table 1 (Skujins and West,

Table 1. Curlew Valley site soil characteristics[a]

Characteristic	Big Sagebrush Site No. 5 0-13 cm depth	Winterfat Site No. 6 0-10 cm depth	Shadscale Site No. 7 0-8 cm depth
Percent sand	8.9	44.9	19.8
Percent clay	25.8	9.2	16.2
Percent silt	65.3	45.9	64.0
Class	Silty-loam	Loam	Silt-loam
ppm B	3.2	0.2	1.0
Na, me/l	308	2.3	64
EC_e, mmhos	43.0	1.7	6.7
Percent $CaCO_3$ (air dry)	17.6	14.0	15.2

[a]From Skujins and West (1972).

1972) and elsewhere (Mitchell, West, and Miller, 1966).

Acetylene reduction assay

The acetylene reduction assay was derived from the method of Stewart, Fitzgerald, and Burris (1967). Soil cores, with intact crust, were moistened to -1/3 bar pressure (water potential) by adding 1.25 ml of distilled water. The soil core tubes were capped with injectable serum bottle rubber stoppers and acetylene (Matheson Co.) was injected to 0.1 atmosphere (0.6 ml C_2H_2). At that concentration of acetylene, competitive inhibition by nitrogen (N_2) is eliminated (Stewart et al., 1971). After reaction, a gas sample was withdrawn with a gas-tight syringe and assayed for ethylene using a Varian series 1700 gas chromatograph with a flame ionization detector. Helium was used as a carrier gas with a flow rate of approximately 25 ml per minute with a 2.74 m Porapak R column, 100 to 120 mesh, at 50 C. The injection temperature was set at 55 C, and the hydrogen flame detector at 90 C. Setting the attenuation of the GC at 1, and the range at 10^{-11}, generally enabled reading of most ethylene peaks. Setting the attenuation at 1, 2, or 4, and range at 10 enabled reading of the acetylene peak which served as an internal standard. Both the attenuation and range could be changed to accommodate any peak reading. Attenuation 1, and range 10^{-11}, was the baseline setting for ethylene determination and standardization.

A Varian model 20 strip chart recorder was used for reading and output of the ethylene and acetylene peaks, with a chart speed

of 15 inches per hour. Peak height is not proportional to sample volume injected so it is important to use the same sample volume for standardization and assay. Gas samples of 0.2 ml were used standardization and assay. Ethylene concentration was read from a standard curve obtained using pure ethylene (matheson Co.).

Soil core assays

Soil cores were assayed as described above.

Crust assays

Soil crust (1 to 2 mm depth) was scraped from the surface of an intact crust so as to cover the bottom surface of a 6.5 ml serum bottle (1.324×10^{-8} hectares). The crust was moistened with 0.25 ml of distilled water, capped, and acetylene injected to 0.1 atmospheres at zero time. After the reaction period, a 0.2 ml gas sample was withdrawn for ethylene analysis.

Soil assays

One-half grams of soil was weighed and placed in 6.5 ml serum bottles. The soils were moistened with 0.25 ml of distilled water and acetylene was injected at zero time. After the reaction period, a 0.2 ml gas sample was withdrawn for ethylene analysis. Separately the percent moisture was determined with a sample of the soil. The soils were dried overnight at 110 C. Percent moisture was determined by weight differences.

Nitrogen fixation calculations

The crust surface area of both the soil core tubes and the serum bottles was 1.324×10^{-8} hectares (based on cross-section or

or bottom surface area of the serum bottles and soil core tubes). The following assumption was made in order to estimate nitrogen (N) fixed by acetylene reduction:

$$C_2H_2 \xrightarrow{2e^-} C_2H_4$$

$$N_2 \xrightarrow{6e^-} 2\,NH_3$$

Therefore, 1.0 nanomole C_2H_4 produced equals 9.33×10^{-9} grams of nitrogen (N) fixed. Multiplying the nanomoles found in the 0.2 ml gas sampled analyzed by 30 yields the total nanomoles produced in an assay (serum bottle or soil core tube). Nanomoles C_2H_4 x .7046 equals grams nitrogen (N) fixed per hectare. To summarize:

$$\text{mm (peak height)} \times \frac{1.5 \text{ nanomoles } C_2H_4}{100 \text{ mm}} \times 30 \times .7046 =$$

grams nitrogen (N) fixed per hectare.

Water potential

Water potential of soils and crusts was determined sith a Wescor (Logan, Utah) psychrometer microvoltmeter MJ55 (Skujins and West, 1973).

Ammonium nitrogen by Kjeldahl method

Soil samples were extracted with 2N KCl (5 ml 2N KCl per gram of soil) by shaking for 0.5 hours. Twenty-five ml of the 2N KCl soil extract was introduced into a Kjeldahl flask, 1 gram of MgO was added, and the mixture boiled by steam. Approximately 40 ml of the distillate was collected in a receiver flask containing 5 ml of 2 percent boric acid and two drops of Tashiro's indicator. The collected solution

was then titrated with standard H_2SO_4. After correction for the KCl blank the following calculation was used:

$$\text{ug } NH_4^+\text{-N/ gram soil} = \frac{\text{ml } H_2SO_4 \times \text{Normality of } H_2SO_4 \times 14.}{5 \text{ grams}}$$

Nitrogen fixation versus light intensity

Unless noted otherwise, all assays incubated in light refers to a fluorescent light intensity of approximately 40 microeinsteins m^{-2} sec^{-1}. Light intensity was measured with a Lambda Quantum sensor (Lambda Instrument Co., Lincoln, Nebraska) with a Wescor microvoltmeter (quantum sensor calibration: 2.52 mv/100 microeinsteins m^{-2} sec^{-1}). The quantum sensor's true response is horizontal for angles less than 82 degrees from the normal axis to the sensor.

Acetylene reduction versus light intensity curves were plotted using acetylene reduction data obtained at 23 C by placing moistened soil cores with intact crusts, collected from the Atriplex confertifolia site, at various distances from the light source. Soil cores were assayed in triplicate for each light intensity. The incandescent light was a Sylvania 200 watt bulb, and the fluorescent light (cool white) consisted of two 4 watt lamps (Stocker and Yale, Inc., Beverly, Mass.). Soil cores incubated under incandescent light required use of a cooling, circulating water bath to maintain the temperature constant.

Nitrogen fixation versus temperature

Unless noted otherwise, all assays were incubated at room temperature (21 ± 2 C).

To assess the effect of temperature upon acetylene reduction, soil cores, collected from the Atriplex confertifolia site, were

moistened, and placed in incubators at temperatures from 4 to 45 C for 12 hours under fluorescent light at 40 microeinsteins m^{-2} sec^{-1} intensity. The moistened soil cores were equilibrated for 1 hour at each temperature before acetylene addition. Assays were run in triplicate at each temperature.

Nitrogen fixation versus water potential

To determine the effect of water potential upon acetylene reduction, "dry" soil cores from the Atriplex confertifolia site were moistened with amounts of water from 1.25 ml to no addition. The cores were incubated at 23 C for 24 hours under fluorescent light at 40 microeinsteins m^{-2} sec^{-1} intensity. After assay for ethylene, the crust was removed from the surface and the water potential measured with a Wescor psychometer and microvoltmeter.

In situ acetylene reduction

In situ assays were run on the Atriplex confertifolia site beginning 8:00 a.m. September 20 and ending 8:00 a.m. September 21, 1973. The 24 hour period was a partially cloudy fall day, typical for the fall in South Curlew Valley. For each reaction period, two soil cores with intact crust were moistened with 1.25 ml of distilled water, capped, and 0.6 ml of acetylene was injected. After the reaction period, a 1.0 ml gas sample was withdrawn and injected into a 6.5 ml serum bottle which was returned to the laboratory for ethylene analysis. For each succeeding reaction period, two soil cores were moistened and treated as above. All of the soil cores were returned to the laboratory on September 21, for assay under laboratory conditions

(23 C, with fluorescent light of 40 microeinsteins m^{-2} sec^{-1} intensity). Air temperature and temperature of a moistened soil crust were measured with a mercury thermometer. Light intensity was measured with the quantum sensor during the reaction periods, and expressed as a mean, since light intensity varied during the reaction periods.

In situ assays were also performed with three soil cores with a reaction period from 11:30 a.m. to 1:00 p.m. on April 14, 1973 as described above.

Canopy effect

Samples taken "under the plant canopy" refer to those soil cores collected within the 20 to 40 cm radius around each plant, where the soil surface is under the influence of the shrub canopy (litter, shading, etc.). Bryophytes tended to dominate the surfaces beneath most shrub canopies, with the absolute area under the canopy varying with shrub size.

Plant extract inhibition

Five grams of plant leaves were ground in 20 ml of water with a mortar and pestle for 5 minutes. The resulting slurry was then filtered through a Whatman No. 1 filter. The mortar was rinsed with 80 ml of distilled water, and the solution was poured and filtered with the plant leaf slurry. Extracts were prepared from leaves of the three desert shrubs mentioned above. Soil cores collected from the Atriplex confertifolia site were moistened with 1.25 ml of the extracts and incubated at 23 C for 24 hours under fluorescent light

at 40 microeinsteins m^{-2} sec^{-1} for the acetylene reduction assay.

Plant leaf-soak filtrates

One gram of plant leaves from each of the three desert shrubs mentioned above was soaked in 10 ml of water overnight and then filtered through a Whatman No. 1 filter. The soil cores were moistened with 1.25 ml of the filtrate prior to the acetylene reduction assay.

DCMU inhibition

Five drops of a 0.01 M DCMU (dichlorophenyl dimethyl urea) aqueous solution were added to moistened soil cores. Cores were first incubated and assayed for acetylene reduction prior to DCMU addition, and then incubated and assayed after DCMU addition.

Soil core nitrogen fixation
and storage at 3 C

Soil cores with intact crusts were assayed as described previously at 1, 2, and 7 days after collection. Moistened cores were stored at 3 C during the seven day period. The results of two and ten hour incubations (acetylene reduction assays) were compared.

Plant leaf volatile inhibitors

To assess the presence of volatile inhibitors from desert shrub leaves a 10 cm piece of rubber tubing was attached to the serum bottle containing the moistened algal crust. The rubber tubing held approximately 1 gram of plant leaves, and was incubated so that only volatile plant products could come into contact with the

crust. This was achieved by placing the leaves in the region of the tubing farthest from the crust. The bottles, with attached tubing, were then treated according to the acetylene reduction procedure described previously.

Volatile inhibitors in Artemisia tridentata aqueous extracts

To assess the presence of volatile inhibitors of nitrogen fixation in A. tridentata extracts, 2 ml of extract was pipetted into a 15 x 135 mm test tube; the 13 x 60 mm soil core tubes (stoppered at the bottom) were placed in the larger tubes; and the larger tubes capped with injectable rubber stoppers. Thus the moistened crust would come into contact with only the vapors from the extract. A Philodendron extract was run as a non-aromatic control. The acetylene reduction assay was then performed in the standard fashion.

Ammonium ion inhibition of nitrogen fixation

Quadruplicate soil core tubes were moistened with 1.25 ml of solutions of ammonium sulfate at: 10, 25, 50, and 100 ug NH_4^+-N per tube (per soil core). The tubes were incubated in the light at 21 C for 24 hours and then assayed for acetylene reduction.

Ar versus N_2 effect on acetylene reduction

Serum bottles containing moistened crust was flushed with a mixture of Ar, O_2, CO_2 (80 %, 20 %, 400 ppm, respectively) for 2 minutes. Serum bottles with moistened crust with a normal air atmosphere were also used. Assays were run in triplicate.

Acetylene (0.6 ml) was injected at zero time. Bottles were incubated in the light for 24 hours at 21 C.

Transects

Soil cores were collected each 10 m for 150 m from each of the three desert shrub communities mentioned previously, on May 12, 1973 and September 5, 1973. Acetylene reduction assays were performed as previously described.

RESULTS AND DISCUSSION

Acetylene reduction assay

In the gas chromatography assay for ethylene and acetylene, the retention time for ethylene was 4.5 minutes, and 5.9 minutes for acetylene. Figure 1 shows the ethylene standard curve.

Figure 2 shows the acetylene reduction assay of two relatively active soil cores with intact crust periodically sampled for ethylene over 25 hours. Both samples had been collected "dry" from the Atriplex confertifolia site, moistened, and assayed in the laboratory two weeks after collection. Both samples show that the rate of acetylene reduction is essentially linear between two and 25 hours. However, the sample collected in October exhibited a slight lag. The fact that there is little or no lag indicates that the blue-green algae begin fixing nitrogen almost immediately after a rainfall or moistening. Ethylene production has been detected within five minutes after moistening a crust with water. The presence or absence of lag may depend upon such factors as endogenous levels of ATP, ammonium ion, or reducing substances. Those factors may in turn be a reflection of the drying conditions. For example, slower drying under lower temperatures and light intensities might have a significantly different effect than rapid drying under higher temperatures and light intensities.

Since assays were linear for at least 25 hours, a sample could be assayed at, for example, 23.8 hours, and activity

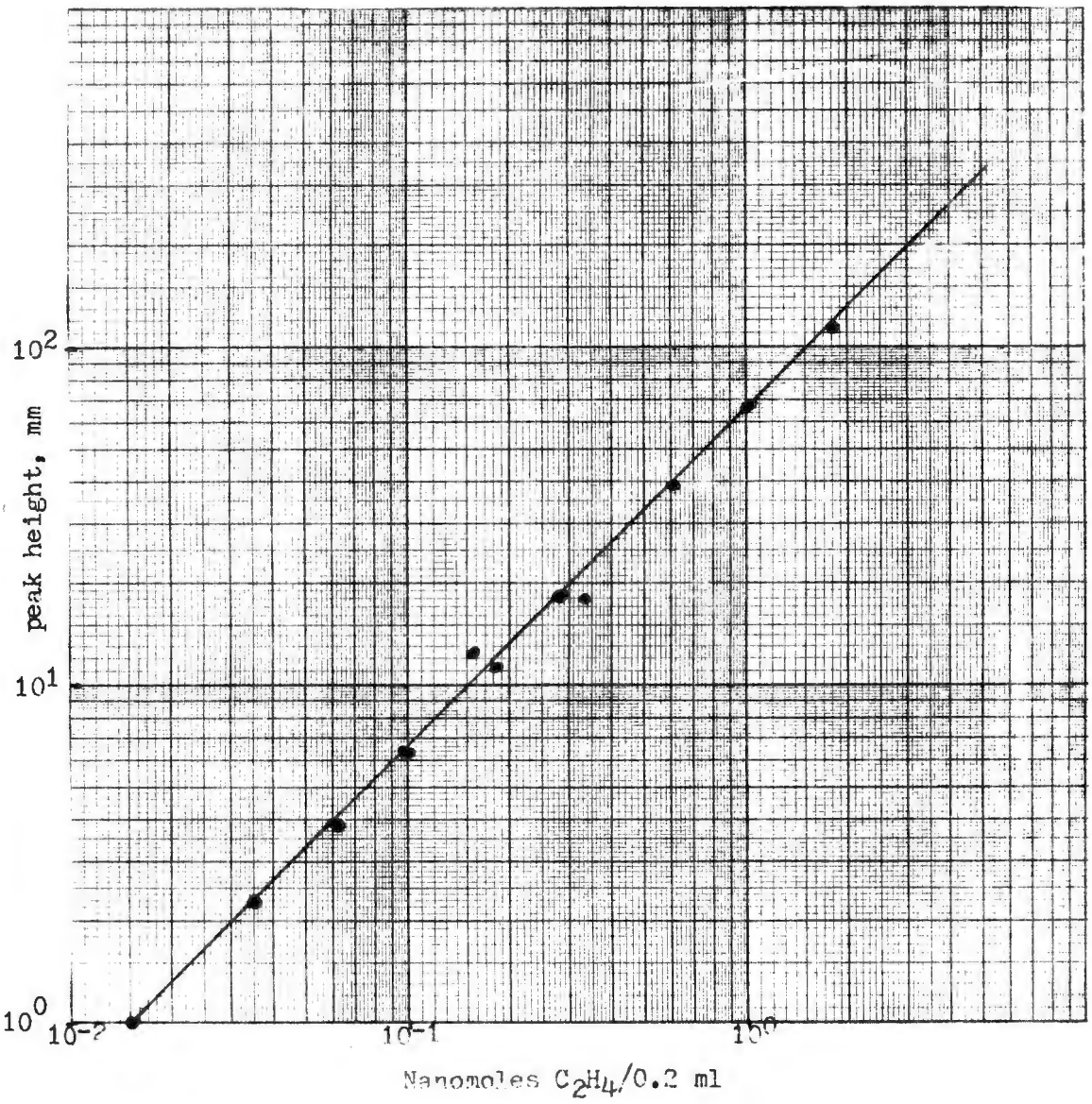

Figure 1. Ethylene standard curve.

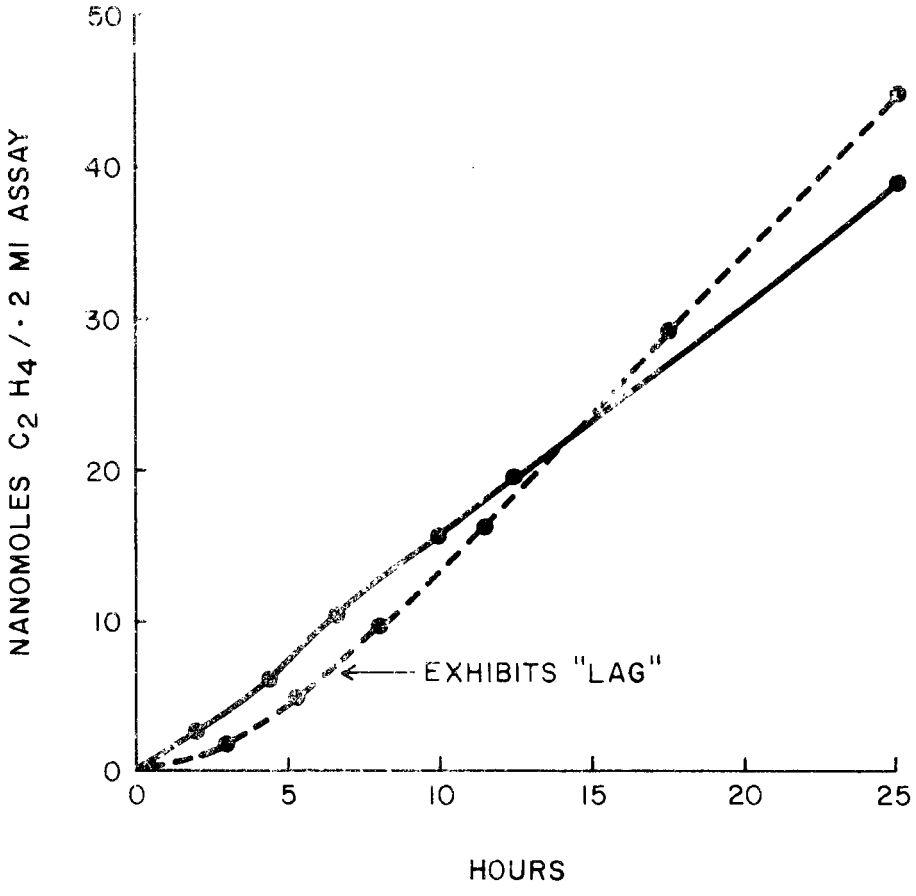

Figure 2. Rate of acetylene reduction. Broken line: soil core collected in October; solid line: soil core collected in July.

extrapolated to 24 hours in order to express activity per 24 hours; or, for example, assay at 24.3 hours, and extrapolate back to get activity per 24 hours. This avoids the complicating use of reagents to stop the nitrogen fixation reaction. In effect, the reaction, for analysis purposes, is stopped when the gas sample is removed from the reaction tube or bottle.

No detectable ethylene was emitted from the soil cores with intact blue-green algae-lichen crusts in the absence of acetylene, and soil beneath the crust did not reduce acetylene (Table 20).

Ar versus N_2

Table 2 shows that there is no difference in soil core acetylene reduction under Ar, O_2, CO_2 versus the natural air atmosphere. This agrees with the results of Stewart et al. (1971).

Loss of activity upon storage at 3 C

Table 3 shows the "loss" of nitrogenase activity upon successive assay and storage at 3 C. One aspect of this loss suggests acetylene toxicity, but 8th day (7th day after collection) 9 hour assays show that the loss of activity is only apparent, dependent upon the length of assay, since about 75 percent of the initial activity was observed with longer reaction incubations. What this implies is that assay and storage of moistened samples at 3 C results in an increased lag period. This points out the importance of assaying moist samples at a standard time after collection, if one wants to compare experiments. If day to day comparisons of activity in experiments are not required, then this loss of activity is not that important.

Table 2. Acetylene reduction as a function of Ar versus N_2 atmosphere

Atmosphere	nanomoles C_2H_4/soil core/hr	SD
Ar, O_2, CO_2	3.56	1.54
N_2, O_2, CO_2	5.15	2.32

Table 3. Loss of activity upon storage at 3 C

Sample[a]	nanomols C_2H_4 ha^{-1} hr^{-1}
1st day, 2-4 hr incubation	62.2
2nd day, "	23.0
3rd day, "	10.9
8th day, "	0.12
8th day, 1st 9 hr incubation	16.6
8th day, 2nd 9 hr incubation	46.8

[a]Site 7 cores collected 26III73.

Nitrogen fixation versus water potential

Figure 3 shows the rapid drop in acetylene reduction as the negative water potential of the blue-green algae-lichen crust increases. Crusts at -13 bars or less exhibited no acetylene reduction. This was somewhat surprising in view of the fact that lichen photosynthesis may occur at relatively low water potentials (-15 bars) (Ahmadjian, 1967). From the water potential data, it would be expected that nitrogen fixation would fall off rapidly after a rainfall as the crust dries. On the other hand, ammonium ion might be effectively concentrated in the crust micro-environment with drying, and ammonium ion probably regulates the level of nitrogenase (Dharmawardene and Stewart, 1972).

Nitrogen fixation versus light intensity

Figure 4 shows the effect of light intensity upon nitrogen fixation. Acetylene reduction reached a maximum at about 200 microeinsteins m^{-2} sec^{-1} with incandescent light, which is a light intensity somewhat comparable to a day with a heavy grey cloud cover (50 to 300 microeinsteins m^{-2} sec^{-1} with natural light). While the spectral composition of fluorescent light, incandescent light, and sunlight is certainly different, the laboratory results with incandescent light yield approximate results, which indicate that relatively low light intensities are required for photosynthesis relative to nitrogen fixation with this system, i.e., nitrogen fixation is maximal at low light intensities.

Fluorescent light results in greater acetylene reduction

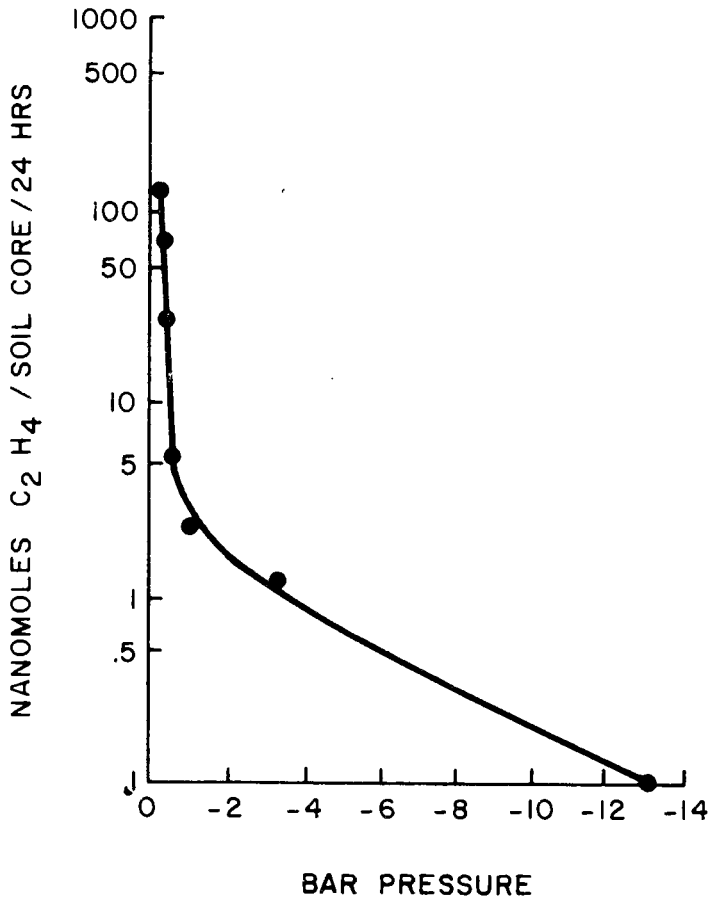

Figure 3. Acetylene reduction versus water potential.

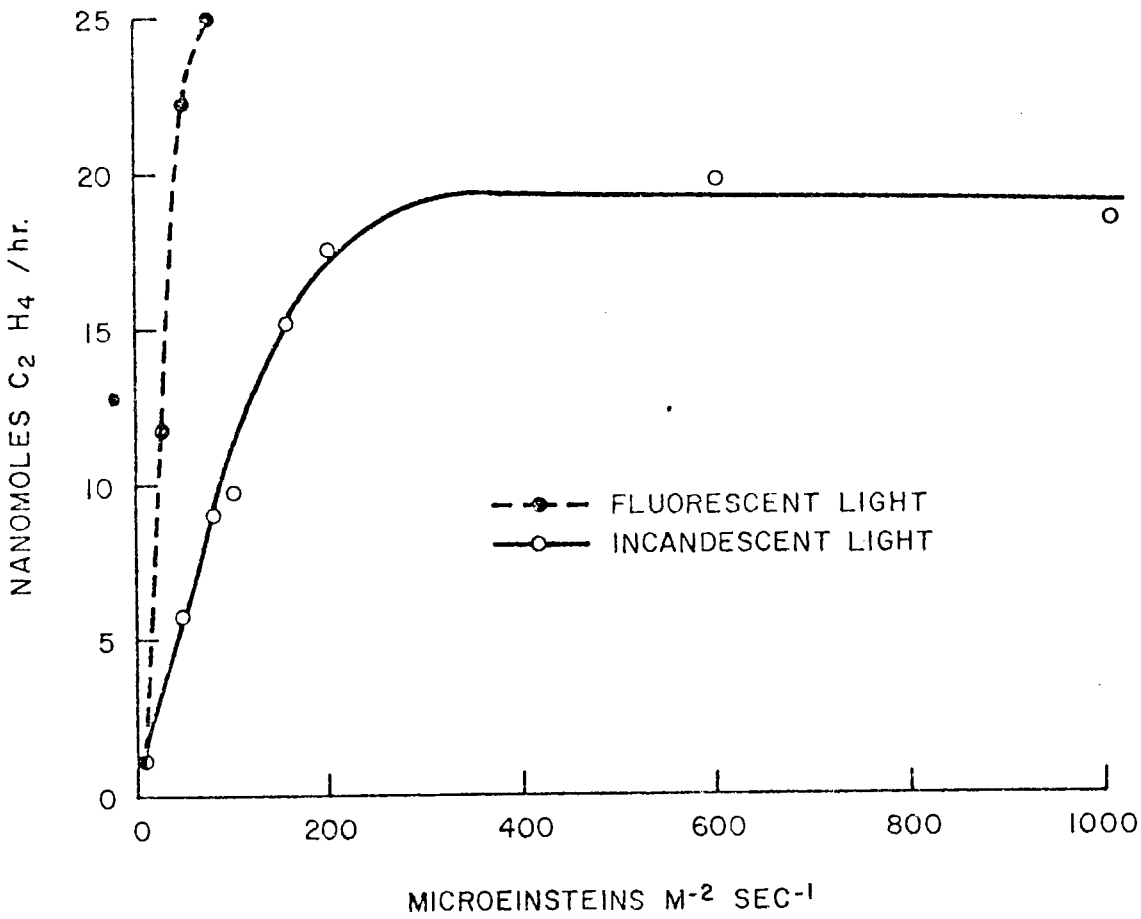

Figure 4. Effect of light intensity upon acetylene reduction.

at the same light intensity than incandescent light. Presumably, the absorbance of light by blue-green algae biliproteins and phycocyanins is the result of this enhancement (Krogmann, 1973). However, fluorescent light was the light source for most assays since little heat was produced.

There is some dark nitrogen fixation shown in Figure 4, approximately 5 percent of the maximum with incandescent light. An evening rainfall could thus be followed by significant dark or night-time nitrogen fixation. A part of the dark nitrogen fixation may be due to bacterial heterotrophic nitrogen fixation within the crust microenvironment. Benemann and Weare (1974) and Henriksson et al. (1972) have reported significant amounts of acetylene reduction by blue-green algae in the dark.

DCMU inhibition

Table 4 shows that DCMU (dichlorophenyl dimethyl urea) can effectively inhibit a good portion of crust nitrogen fixation. The lack of complete inhibition has a number of implications. Photosynthesis is possibly not tightly coupled to nitrogen fixation, and/or mycobiont respiration may reduce the oxygen tension within the crust microenvironment. Stewart (1973) has commented upon the lack of complete inhibition of nitrogen fixation by DCMU. The presence of nitrogenase in the heterocysts of blue-green algae probably partitions nitrogen fixation and photosynthesis. Thus one might expect limited DCMU inhibition, however, blue-green algal acetylene reduction is 90 percent inhibited by DCMU under anaerobic conditions (Benemann and

Table 4. Inhibition of nitrogen fixation by DCMU

Sample conditions[a]	nanomoles C_2H_4 ha^{-1} hr^{-1}		Percent inhibition
	1st 8-10 hr incubation	2nd 8-10 hr incubation[b]	
Light (control)[c]	16.6	46.8	--
Light	10.1	3.40	64
Light/2% glucose	26.3	6.60	73
Dark	0.05	0.00	100
Dark/2% glucose	1.45	0.15	90

[a]Site 7 cores.
[b]DCMU added at zero time.
[c]No DCMU added.

Weare, 1974). These results do suggest that photosynthesis does supply, in great part, the driving energy for nitrogen fixation.

Dark nitrogen fixation

Dark nitrogen fixation was 5 to 30 percent of nitrogen fixation in the light (Figure 4, Table 5, Table 16). Presumably photosynthesis supplies the energy (ATP) and reductant necessary for dark nitrogen fixation. Table 5 shows that moisteneing and pre-incubating soil cores with crusts in the light results in much greater fixation in the dark, when compared with cores moistened and pre-incubated in the dark and subsequently assayed in the dark. The lower nitrogen fixation with samples moistened and pre-incubated in the dark is evidence of loss of reductant due to dark respiration. Samples that were not pre-incubated exhibited nitrogen-fixing activity slightly greater than the samples moistened and pre-incubated in the light, indicating that the state of the blue-green algal-lichen crust (collected "dry") had a significant pool of ATP and reductant so that should an evening or night-time rain occur, there would follow substantial nitrogen fixation. In effect then, the crust was relatively "primed" for nitrogen fixation should moisture become available.

Nitrogen fixation versus temperature

Figure 5 shows the acetylene reduction and temperature relationship based upon laboratory assays. Optimum acetylene reduction occurred at 19 to 23 C. This would imply that there would be little nitrogen fixation during the winter months even

Table 5. Dark nitrogen fixation

Treatment	Nanomoles C_2H_4/soil core/ 24 hours[a]
Moistened and preincubated in dark for 18 hours	6.0
Moistened and preincubated in light for 18 hours	17.6
Moistened at zero time	21.9
Moistened at zero time and incubated in light	79.7

[a]Site 7 cores; means of quadruplicate assays.

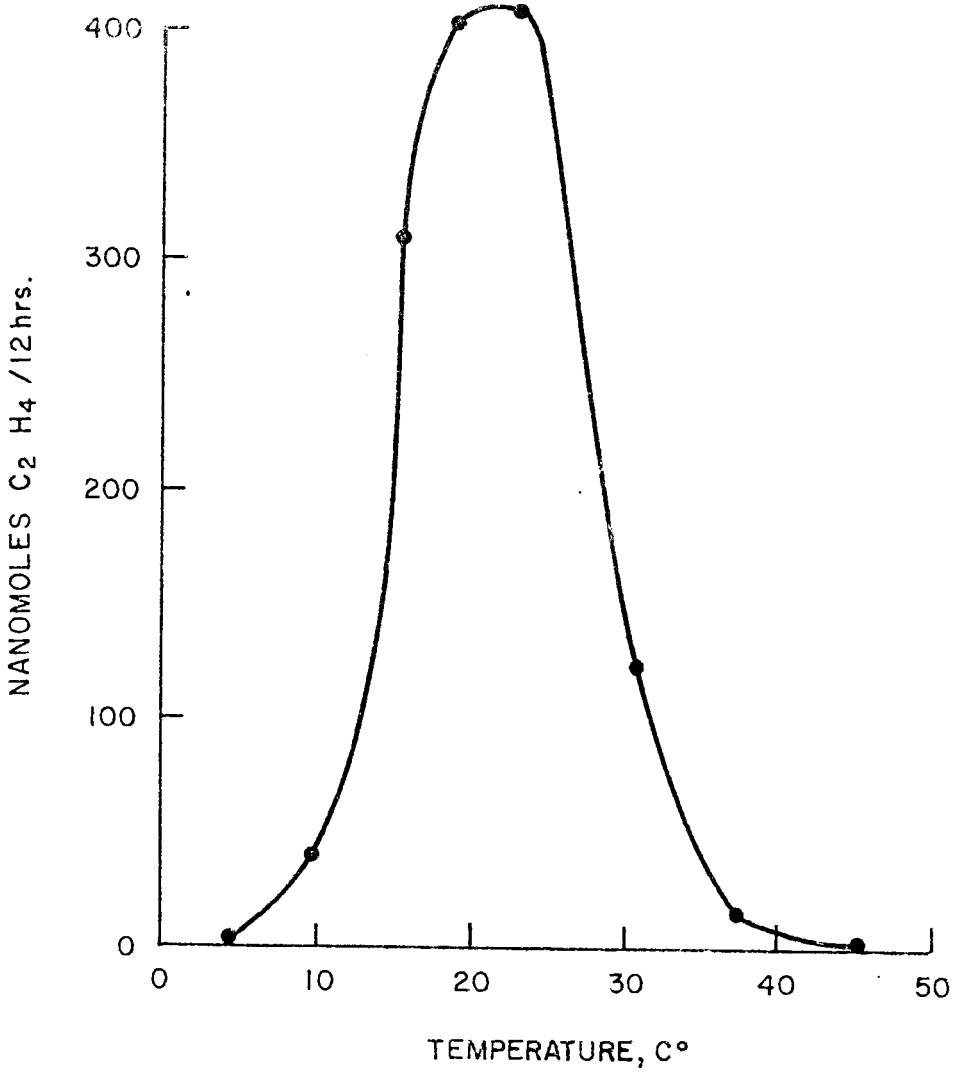

Figure 5. Effect of temperature upon acetylene reduction in the light.

though sufficient moisture might be available. The high temperatures of the summer months (June to September), with soil surface temperatures to 60 C or more, would also limit nitrogen fixation. However, during the summer, surface crust moisture limitations are associated with high ambient temperatures and high light intensities. Thus the greatest amount of nitrogen fixation would occur during the fall and spring when temperatures are moderate and generally within the range shown in Figure 5.

In situ acetylene reduction

Figure 6 shows the results of the September in situ assays. Nitrogen fixation followed mean light intensity and temperature. Air temperature and reaction tube temperature never varied by more than two degrees C. Ninety grams of nitrogen (N) was fixed per hectare in the 24 hour period, assuming 100 percent crust cover. Approximately one gram of N per hectare was fixed during the night. When the soil cores that had been assayed in situ, were subsequently assayed in the laboratory, nitrogen fixation values varied from 75 to 84 g N fixed ha^{-1} hr^{-1}. Even peak values in the field (10 to 14 g N fixed $ha^{-1}hr^{-1}$) were only 13 to 18 percent of their laboratory values (Table 6).

In situ assays for April and peak values for September are shown in Table 6. The percent of the nitrogen fixation potential (laboratory assay) predicted from the temperature curve (Figure 5) gave results which were in good agreement with the in situ data, that is, with the in situ assays' percent values of the laboratory assays. Since light and moisture were not limiting then prediction

Figure 6. *In situ* acetylene reduction.

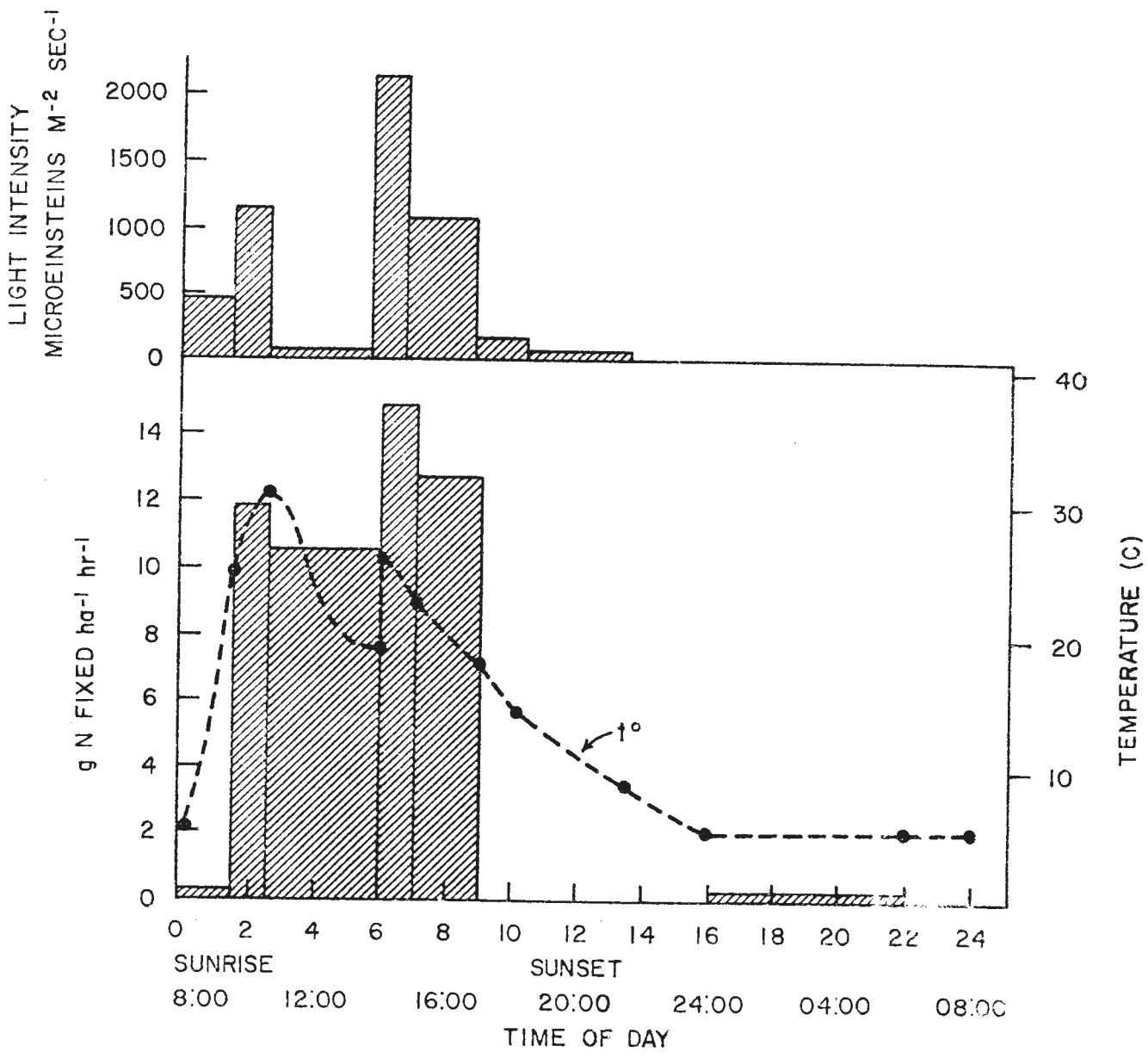
LIGHT INTENSITY
MICROEINSTEINS M-2 SEC-1
2000
1500
1000
500
0
g N FIXED ha-1 hr-1
14
12
10
8
6
4
2
0
40
30
20
10
TEMPERATURE (C)
1°
0 2 4 6 8 10 12 14 16 18 20 22 24
SUNRISE
SUNSET
8:00 12:00 16:00 20:00 24:00 04:00 08:00
TIME OF DAY

Table 6. Comparison of *in situ* and laboratory acetylene reduction on soil cores from the *Atriplex confertifolia* site

Season of Assay	Nitrogen fixed g/ha/hr		Temp C° in situ	Percent activity of potential	
	In situ	laboratory (potential)		in situ	predicted (Fig. 5)
April[a]	11.4	66.6	18	17	17
September[b]	10.5	80.8	20	13	13
September[b]	14.5	80.6	26	18	23
September[b]	12.6	84.0	16	15	15

[a]Mean of triplicate assays.
[b]Mean of duplicate assays.

based upon temperature is possible. This would suggest that during much of the fall and spring rainy seasons, when the blue-green algae-lichen crust is wet, that it is largely temperature that governs the rate of nitrogen fixation. However, during the hot, dry summer months, moisture availability would limit nitrogen fixation.

Crust heterotrophic nitrogen fixation versus temperature

Figure 7 shows that crusts, moistened with 2 percent glucose solutions and incubated in the dark, exhibited a temperature optimum of about 30 c for nitrogen fixation. Thus heterotrophic nitrogen fixation exhibits an optimum 10 degrees C higher than crust autotrophic nitrogen fixation. This shift may reflect the potentiation of Azotobacter and Clostridium nitrogen fixation by glucose in the crust micro-environment. Another possibility is that glucose stimulates mycobiont respiration, and thus autotrophic and/or heterotrophic nitrogen fixation is enhanced, perhaps by oxygen tension reduction.

Glucose potentiation of nitrogen fixation

Table 7 shows that glucose can potentiate nitrogen fixation by crust in the light. Glucose can also potentiate nitrogen fixation of soils in the dark. Without glucose addition, there is little or no heterotrophic nitrogen fixation in soils. Thus there is a heterotrophic nitrogen fixation potential in crusts and soils beneath the crust, but nitrogen fixation is probably nil or negligible in situ due to low carbohydrate availability.

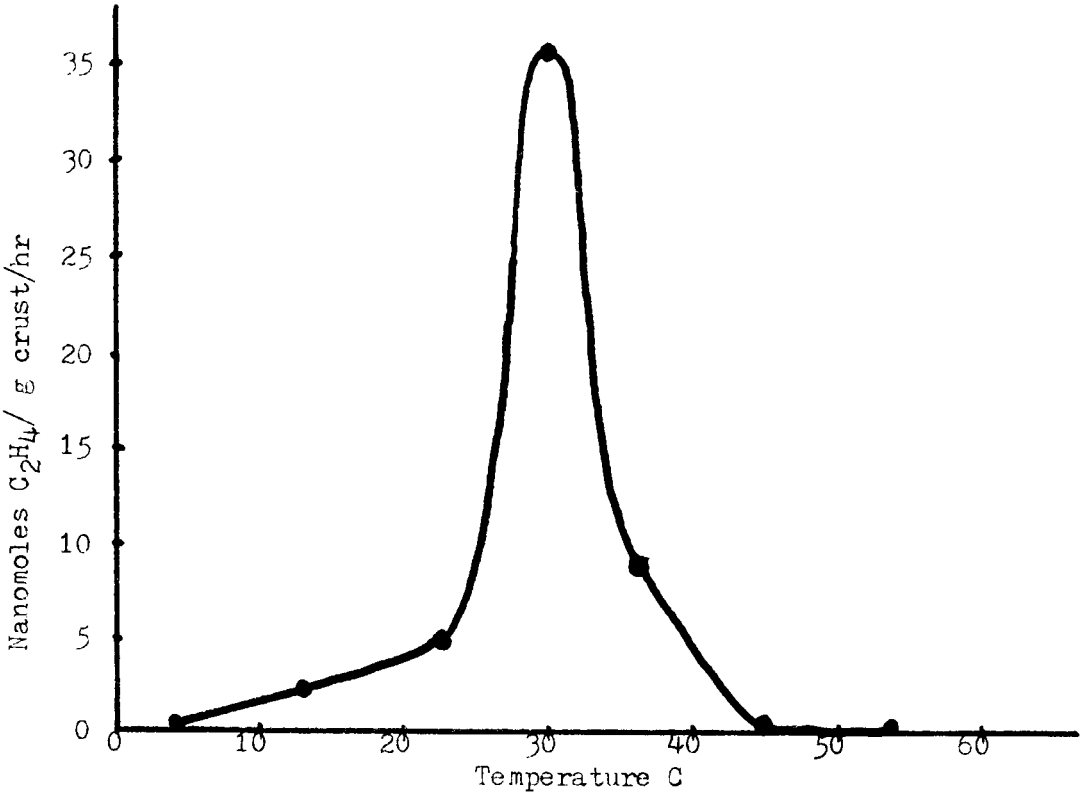

Figure 7. Effect of temperature upon crust heterotrophic nitrogen fixation.

Table 7. Glucose potentiation of nitrogen fixation

Sample	Conditions	Activity: nanomoles C_2H_4/g soil[a] 24 hours	48 hours	70 hours
5-crust	Light	9.9	65.0	
6-crust	Light	0.11	0.22	
7-crust	Light	2.1	1.7	
5-crust	light/glucose	7.4	235.0	
6-crust	Light/glucose	0.46	10.0	
7-crust	Light/glucose	170.0	710.0	
5: 0-3 cm	Dark/glucose	0.0	17.5	
6: 0-3 cm	Dark/glucose	0.0	10.5	
7: 0-3 cm	Dark/glucose	6.3	290.0	
5: 5-20 cm	Dark/glucose	0.0	5.9	32.0
6: 5-20 cm	Dark/glucose	0.0	0.15	3.3
7: 5-20 cm	Dark/glucose	0.0	0.45	2.0

[a]Mean of duplicate assays.

Seasonal effects

Figure 8 shows the laboratory nitrogen fixation potential results of some acetylene reduction assays (means of triplicate assays plotted) of soil cores with intact crusts from the Atriplex confertifolia site. While it is difficult to draw conclusions from these few assays, a few implications may be present. Not all of the assays were performed on the same day or days after collection. Table 8 shows the dates of collection and assay and activity (nitrogen fixation) of the samples plotted in Figure 8. There does not appear to be a significant year to year change in the nitrogen fixation potential, particularly evidenced by the June '73 and June '74 values being relatively similar. However, there appear to be fall and spring peaks, which might be expected since the rainy periods occur then in the Great Basin Desert.

Diurnal fluctuations in nitrogen fixation occur (Balandreau, Millier, and Dommergues, 1974), and Figure 6 implies this as well. Thus a diurnal fluctuation has a long term seasonal fluctuation superimposed.

Table 9 shows the results of the transect sample assays from each of the three desert shrub communities for the spring and early fall. In this case, spring and fall values are not significantly different. May and September results are averaged for later use in estimating site nitrogen input by nitrogen fixation. The Ceratoides values are significantly lower and reflect the scant lichen cover in that shrub community. The Artemisia and Atriplex values are not significantly different. These results suggest that May through September nitrogen fixation potentials do not change much. This

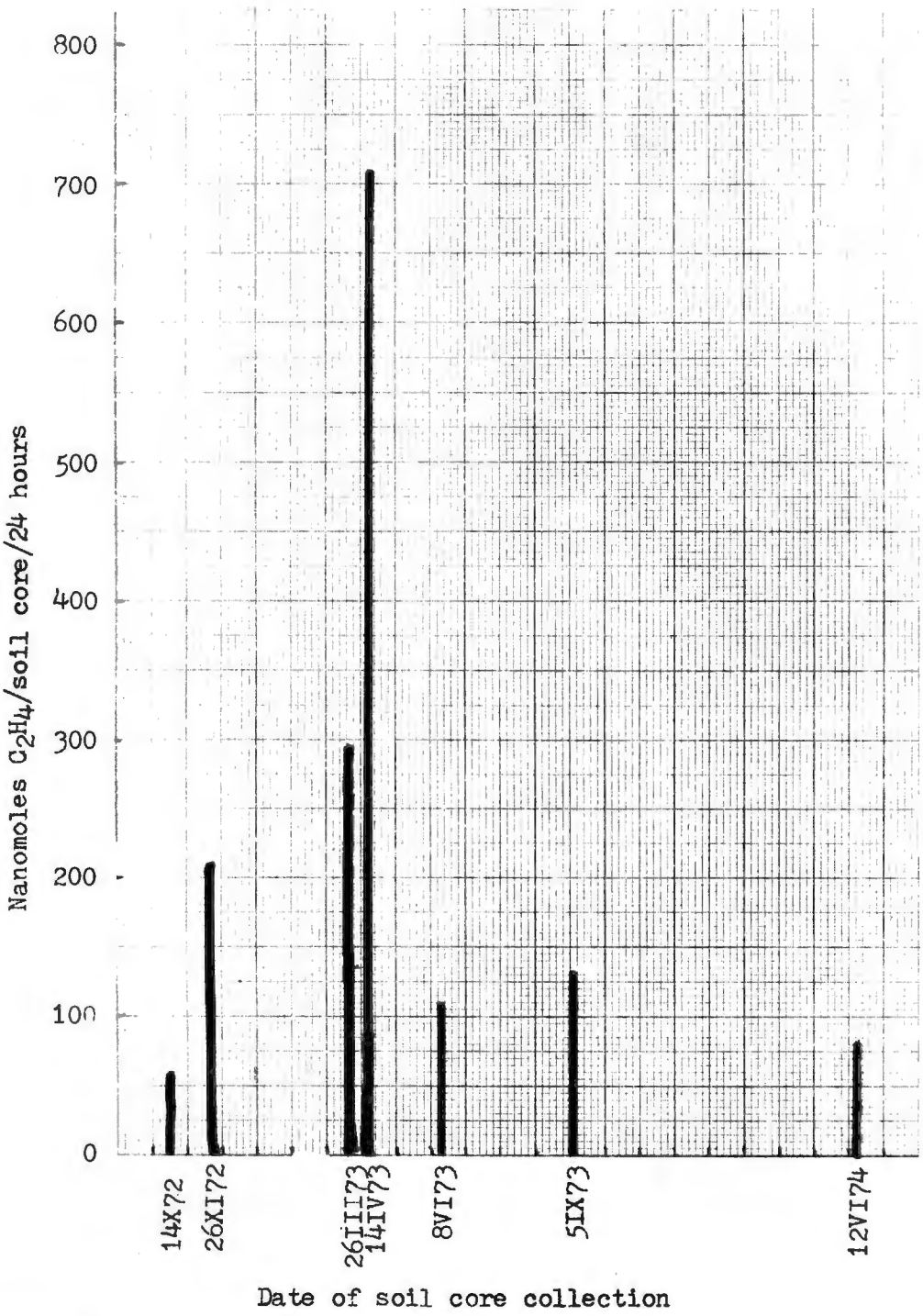

Figure 8. Nitrogen fixation seasonal effect.

Table 8. Nitrogen fixation: seasonal effect

Date collected[a]	Date of assay	Nanomoles C_2H_4/soil core/24 hrs[b]
14X72	27XII72	58.5
26XI72	28XI72	212.4
26III73	21V73	297.0
14IV73	26IV73	720.0
8VI73	13VI73	108.0
5IX73	11II74	132.8
12VI74	14VI74	85.5

[a]Site 7 soil cores.
[b]Mean of triplicate assays.

Table 9. Acetylene reduction potential of site transect samples

Site	grams N fixed/ha/ 24 hours[a]				
	May	SD	September	SD	May-September mean
Atriplex	106.	102.	151.	280.	128.5
Ceratoides	3.5	6.5	4.7	5.5	4.1
Artemisia	156.	188.	64.8	83.8	110.4

[a]Mean of 15 soil cores.

might be expected since little rain occurs during this period. With slow growth rates for lichens, one might expect little year to year difference in transect assays.

For desert ecosystems, no knowledge is yet available as to lichen growth rates, or stability of the crust in terms of disturbance.

Nitrogen fixation versus soil characteristics

Site transect nitrogen fixation (Table 9) suggests that perhaps the low percent sand and low Boron concentration of soil from the Ceratoides lanata site (Table 1) are related to the poor crust development. However, the transect nitrogen fixation and soil data are insufficient to draw any definite conclusions, and soil micro-enviornment characteristics are not available.

Estimates of nitrogen input

Spring in situ assays showed that 90 grams of nitrogen (N) was fixed per hectare in 24 hours (Figure 6). Based upon 120 days of fixation (fall and spring) at that rate, 10.8 Kg N could be fixed.

Up to 14 grams N may be fixed ha^{-1} $hour^{-1}$ in situ (Figure 6). Based upon 120 days of fixation (12 hours per day) at that rate, 18.7 Kg of N could be fixed annually.

The nitrogen fixation potential (laboratory assays) could be as high as 80 grams N ha^{-1} $hour^{-1}$ (Table 6). Based upon 120 days of fixation (12 hours per day) at that rate, 115.2 Kg N could be fixed annually. The previously discussed estimates would be for areas of 100 percent crust cover. Thus an estimate of 10 to 100

Kg N fixed ha^{-1} $year^{-1}$ might be a reasonable one.

Based upon 120 days of nitrogen fixation at the May-September mean rate shown in Table 9, up to 7.7 Kg N ha^{-1} could be fixed on the Atriplex confertifolia site; up to 0.25 Kg N ha^{-1} on the Ceratoides lanata site; and up to 6.6 Kg N ha^{-1} on the Artemisia tridentata site. Assuming that the transects provide a rough estimate of nitrogen-fixing crust cover, then annual nitrogen input, at least on the Atriplex site, would be toward the lower end of the 10 to 100 Kg N range.

Nitrogen input by crust nitrogen fixation depends then upon the extent of crust cover, rainfall and temperature during the fall and spring rainy seasons, and duration of the rainy periods.

Ammonium ion inhibition of nitrogen fixation

Exogenously supplied NH_4^+-N can effectively inhibit nitrogen fixation in 24 hour incubation assays (Figure 9). Ammonium ion probably regulates the level of nitrogenase in blue-green algae (Dharmawardene and Stewart, 1972). No knowledge is yet available as to the flux of ammonium ion in the crust micro-environment but presumably the level of ammonium ion could play some regulatory role in blue-green algae-lichen crust nitrogen fixation.

Desert shrub canopy effect

Nitrogen fixation was reduced under all the desert shrub canopies (Table 10). The environment under the shrub canopies is largely dominated by bryophytes. The canopy effect also involves thermal radiation effects, moisture regimes, and litter organic matter. So the reduction of nitrogen fixation under the desert

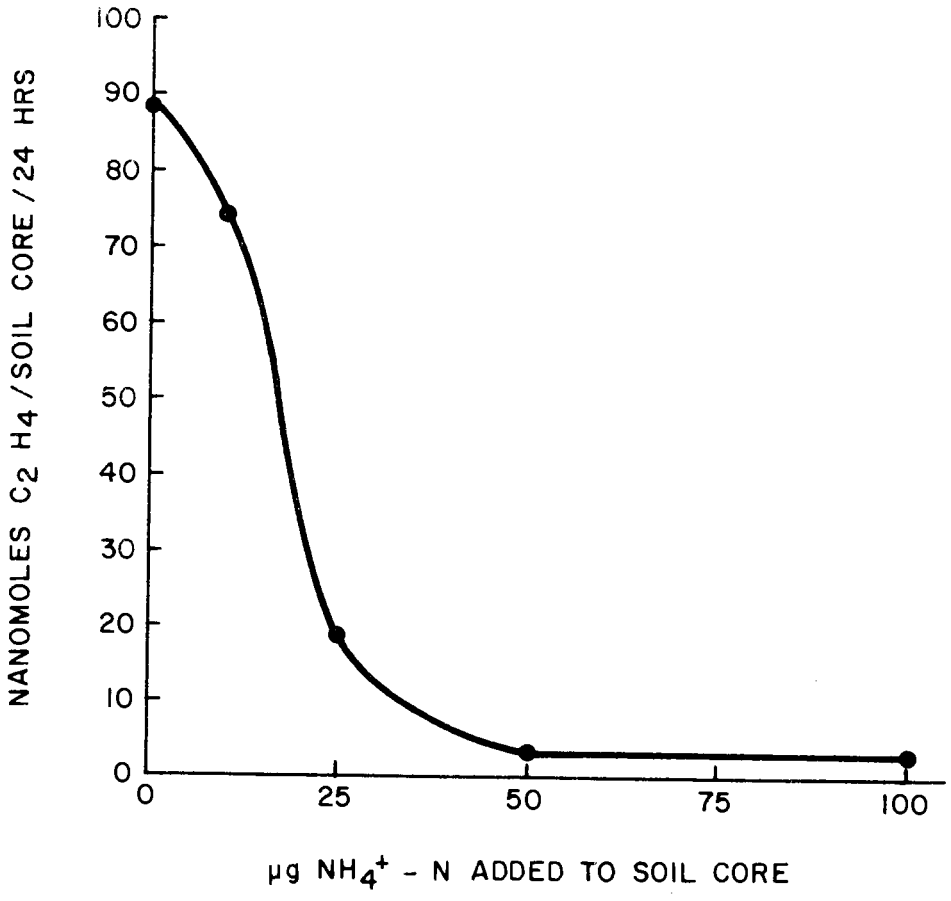

Figure 9. Ammonium ion inhibition of nitrogen fixation.

Table 10. Effect of desert shrub canopy upon acetylene reduction

Site	Nanomoles C_2H_4/soil core/24 hours[a]	
	Interspace	Under canopy
Atriplex confertifolia	78.7	20.7
Ceratoides lanata	4.5	2.3
Artemisia tridentata	79.5	10.8

[a]Mean of triplicate assays.

shrubs is due to a number of factors, but is an important aspect governing the input of nitrogen into the desert shrub communities.

Exchangable ammonium ion and clay and organic matter-fixed ammonium ion do not appear to be signficantly different under the desert shrub canopies versus the interspaces (Tables 11 and 12).

Shrub leaf extract inhibition of nitrogen fixation

Table 13 shows the effect of desert shrub leaf aqueous extracts upon algal-lichen crust nitrogen fixation. All of the extracts possess apparent inhibitors of acetylene reduction, with the leaves of Artemisia tridenata being particularly potent. The canopy effect involves many factors, but it is possible that desert shrub inhibitors are responsible for the scarcity of algal-lichen crusts, and dominance of bryophytes beneath the canopy. Microscopically, Nostoc have been observed in association with some of the lichen thalli. Significant acetylene reduction was never seen in the absence of lichens.

Some of the inhibition by extracts may be due to ammonium ion in the extracts, but this could not account for all of the inhibition (Table 14).

Leaf soak filtrates

Leaf soak filtrates also showed signficant inhibition of nitrogen fixation (Table 15). Perhaps plant leachate and rain-wash of plants and litter can exert an inhibitory effect upon nitrogen fixation.

Table 11. KCl extractable NH_4^+-N: interspace versus canopy

Sample[a]	NH_4^+-N µg/g soil
5-interspace	3.6
5-interspace, no crust	1.5
5-under canopy	2.9
6-interspace	2.4
6-under canopy	2.2
7-interspace	0.0
7-interspace, no crust	0.0
7-under canopy	0.0

[a]0-3 cm soil samples collected 12V73.

Table 12. Clay and organic matter fixed NH_4^+-N; interspace versus canopy

Sample[a]	NH_4^+-N µg/ g soil
5 - interspace	29.8
5 - interspace, no crust	50.6
5 - under canopy	46.9
6 - interspace	39.4
6 - under canopy	25.7
7 - interspace	30.9
7 - interspace, no crust	39.7
7 - under canopy	37.3

[a]0-3 cm soil samples collected 12V73; kjeldahl distillation with 5 ml 40 percent NaOH.

Table 13. Inhibition of nitrogen fixation by desert shrub leaf extracts

Extract	Nanomoles C_2H_4/soil core/ 24 hrs[a]
Control (water)	24.8
Artemisia tridentata	0.0
Ceratoides lanata	5.2
Atriplex confertifolia	8.[illegible]

[a]Mean of triplicate assays.

Table 14. Ammonium ion in 5 percent extracts of desert shrub leaves

Plant	μg NH_4^+-N/ ml extract
Atriplex confertifolia	12.3
Ceratoides lanata	9.4
Artemisia tridentata	7.3

Table 15. Inhibition of nitrogen fixation by desert shrub leaf soak filtrates

Filtrate	Nanomoles C_2H_4/soil core/hr[a]
Control (water)	25.6
Artemisia tridentata	1.1
Ceratoides lanata	16.3
Atriplex confertifolia	14.7

[a]Mean of triplicate assays.

Inhibition of autotrophic and heterotrophic nitrogen fixation by Artemisia tridentata aqueous extracts

Aqueous extracts (1 percent) of A. tridentata leaves can signficantly inhibit autotrophic nitrogen fixation (approximately 65 percent inhibition) and heterotrophic (glucose potentiated) nitrogen fixation (approximately 69 percent inhibition) (Table 16).

Leaf volatile inhibitors

Volatile products from the leaves of all three desert shrubs examined can inhibit nitrogen fixation (Tables 17 and 18). Figure 10 shows diagramatically how the volatile inhibition was assayed. Thus, volatile chemicals from the leaves of the shrubs may, for example, inhibit nitrogen fixation in the crust micro-environment, although this effect would be difficult to demonstrate in situ. The non-aromatic Philodendron species showed no inhibition.

Extract volatile inhibitors

Even the 5 percent extract of A. tridentata exhibited some inhibition of nitrogen fixation (Table 19). An extract of the non-aromatic Philodendron showed no inhibition. Figure 11 shows diagramatically how the aqueous extract volatile inhibitors were assayed.

Potentiation and inhibition of heterotrophic nitrogen fixation

Soils, moistened with water, exhibited no measurable heterotrophic nitrogen fixation, but upon moistening soils with 10 percent glucose solutions there was a significant potentiation of hetero-

Table 16. Inhibition of autotrophic and heterotrophic nitrogen fixation by 1 percent extracts of A. tridentata

Treatment	Nanomoles C_2H_4/soil core/24 hours[a]
Dark incubation	23.9
Dark incubation/extract	20.3
Light incubation	79.7
Light incubation/extract	28.4
Dark incubation/glucose	1[illegible]7.5
Dark incubation/glucose/extract	40.5

[a]Site 7 soil cores; mean of triplicate assays.

Table 17. Inhibition of nitrogen fixation by desert shrub leaf volatile products

Desert shrub leaves	Nanomoles C_2H_4/soil core/18 hrs[a]
Control (no leaves)	22.9
Artemisia tridentata	9.3
Ceratoides lanata	9.9
Atriplex confertifolia	10.2

[a]Site 7 soil cores; calculation based on soil core gas volume of 6 ml.

Table 18. Inhibition of nitrogen fixation by desert shrub leaf volatile products

Desert shrub leaves	Nanomoles C_2H_4/soil core/48 hrs[a]
Control (no leaves)	318.2
Philodendron species	432.7
Artemisia tridentata	54.2
Ceratoides lanata	65.8
Atriplex confertifolia	59.0

[a]Site 7 soil cores.

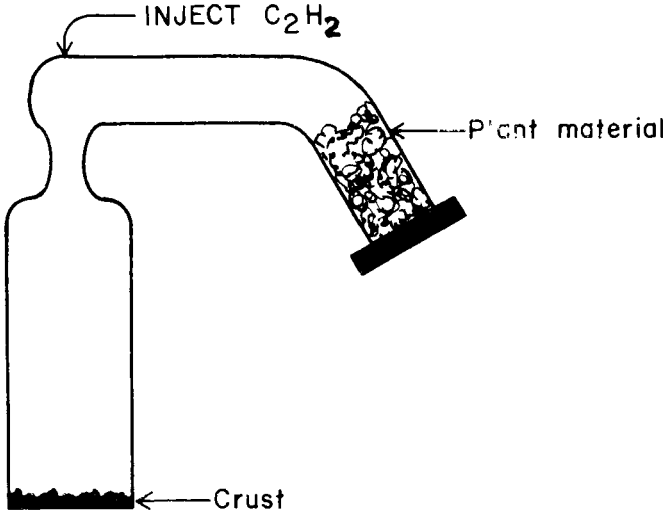

Figure 10. Leaf volatile inhibitor experimental set-up.

Table 19. Inhibition of nitrogen fixation by volatile products from *Artemisia tridentata* aqueous extracts

Sample	Nanomoles C_2H_4/soil core/36 hrs[a]
Control (water)	297.
Philodendron species	289.
Artemisia tridentata	202.

[a]Site 7 soil cores; calculation based on soil core gas volume of 6 ml.

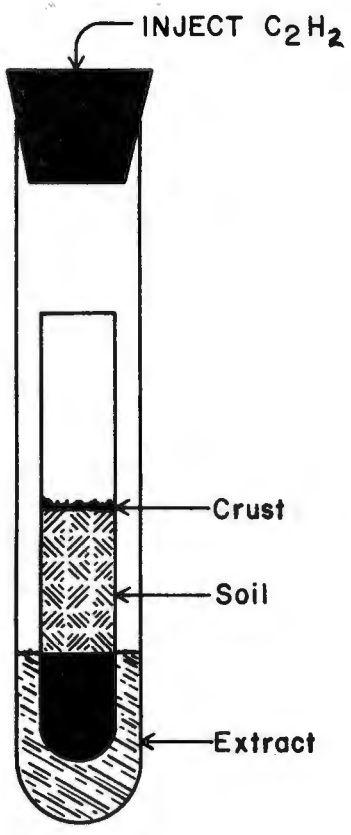

Figure 11. Aqueous extract volatile inhibitor experimental set-up.

trophic nitrogen fixation (Table 20). Soils at the 40 to 50 cm depth exhibited no measurable potentiation of heterotrophic nitrogen fixation. Soils (0 to 3 cm depth) collected from beneath plant canopies, exhibited a greatly reduced heterotrophic nitrogen fixation potential compared to interspace samples. This implies that the heterotrophic nitrogen-fixing population, although low or very dormant in interspace soils, is even lower in soils beneath the shrub canopies. This indirectly supports the notion of plant microbial inhibitors.

Addition of dried leaves to soils, and moistening with water, resulted in a small potentiation of heterotrophic nitrogen fixation, with the leaf organic compounds or decomposition products presumably providing the carbon source for heterotrophic nitrogen fixation. However, dried plant leaves with soils moistened with 10 percent glucose solutions resulted in a significant inhibition of heterotrophic nitrogen fixation (as potentiated by glucose). This demonstrates that shrub material can inhibit heterotrophic nitrogen fixation.

Table 20. Potentiation and inhibition of heterotrophic nitrogen fixation

Site	Soil Sample	Ammendment[a]	nanomoles C_2H_4/gram soil, in 3 days at 30C
Artemisia tridentata	0-3 cm	--	0.0
	0-3 cm	glucose	1760.7
	0-3 cm, under canopy	glucose	4.5
	5-20 cm	--	0.0
	5-20 cm	glucose	27.0
	40-50 cm	--	0.0
	40-50 cm	glucose	0.0
Ceratoides lanata	0-3 cm	--	0.0
	0-3 cm	glucose	5740.7
	0-3 cm, under canopy	glucose	296.1
	5-20 cm	--	0.0
	5-20 cm	glucose	458.8
	40-50 cm	--	0.0
	40-50 cm	glucose	0.0
Atriplex confertifolia	0-3 cm	--	0.0
	0-3 cm	glucose	1696.5
	0-3 cm, under canopy	glucose	90.0
	5-20 cm	--	0.0
	5-20 cm	glucose	4.8
	40-50 cm	--	0.0
	40-50 cm	glucose	0.0
	0-3 cm	Artemisia leaves	2.7
	0-3 cm	Ceratoides leaves	6.3
	0-3 cm	Atriplex leaves	2.7
	0-3 cm	Artemisia leaves/glucose	3.6
	0-3 cm	Ceratoides leaves/glucose	4.5
	0-3 cm	Atriplex leaves/glucose	3.6

[a]0.5 g soil moistened with 0.25 ml water or 10 percent glucose solution; 200 mg dried plant material/0.5 g soil.

SUMMARY AND CONCLUSIONS

The acetylene reduction assay was used to measure nitrogen fixation by blue-green algae-lichen crusts from South Curlew Valley Utah, in the Great Basin Desert.

Crust nitrogen fixation dropped rapidly below -1/3 bar pressure (water potential), and exhibited a temperature optimum of 19 to 23 C, under fluorescent light (40 microeinsteins m^{-2} sec^{-1}). Nitrogen fixation reached a maximum at 200 microeinsteins m^{-2} sec^{-1} of incandescent light intensity, a relatively low light intensity. Some dark nitrogen fixation was observed, varying from 5 to 30 percent of light nitrogen fixation. These laboratory assays suggested that nitrogen fixation occurs primarily during the fall and spring rainy seasons in the Great Basin Desert. Low temperatures would limit nitrogen fixation in the winter, and the low level of moisture would limit nitrogen fixation during the hot, dry summer months.

In situ and laboratory acetylene reduction assays were compared. Nitrogen fixation potential values (laboratory assays) as high as 84 g N fixed ha^{-1} hr^{-1} were obtained. The highest in situ values (14 g N fixed ha^{-1} hr^{-1}) were 13 to 18 percent of their potentials in the laboratory. Since light and moisture were not limiting in the in situ assays, nitrogen fixation agreed with the values predicted from the laboratory temperature curve. An estimate of 10 to 100 Kg N fixed annually was made, based upon 120 days of fixation. This estimate would vary depending upon the extent of nitrogen-

fixng crust cover.

There appeared to be fall and spring peaks in the nitrogen-fixing potential of crusts, which correlates with the rainy seasons.

Soil core transect samples from the Atriplex confertifolia, Ceratoides lanata, and Artemisia tridentata sites showed that there was not a significant difference in the nitrogen-fixing potential between May and September transects. The Ceratoides lanata potential was significantly lower, and reflected the scant lichen cover in that shrub community.

Glucose was found to potentiate heterotrophic nitrogen fixation of moistened crusts and soils. The crust heterotrophic temperature optimum was found to be 30 c. No ethylene was produced in the absence of glucose. Presumably, in the field, the amount of available organic carbon is too low to significantly potentiate heterotrophic nitrogen fixation.

Blue-green algae-lichen crusts were found to be greatly reduced under the desert shrub canopies of Atriplex confertifolia, Ceratoides lanata, and Artemisia tridentata. Leaf aqueous extracts and leaf volatile products inhibited crust autotrophic nitrogen fixation. Dried shrub leaves inhibited glucose potentiated, soil heterotrophic nitrogen fixation. The heterotrophic nitrogen-fixing potential was reduced in soils collected from beneath shrub canopies. Shrub inhibitors of nitrogen fixation may then play a role in nitrogen input in desert shrub communities.

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VITA

Robert Charles Rychert

Candidate for the Degree of

Doctor of Philosophy

Dissertation: Nitrogen Fixation in Arid Western Soils

Major Field: Biology-Ecology

Biographical Information:

Personal Data: Born in Pittsburgh, Pennsylvania, January 19, 1943.

Education: Attended St. Francis Xavier elementary school in Pittsburgh, Pennsylvania; graduated from North Catholic High School in 1960; received the Bachelor of Science degree from Cornell University, with a major in Bacteriology, in 1965; received a Master of Arts degree in Biology from San Francisco State College in 1968.